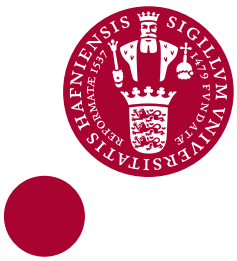

Hyper- and hypo-biofilm forming mutants of Listeria monocytogenes G (Serotype 4b)

Huang, Yanyan; Zhu, X.; Shi, X.; Knøchel, Susanne

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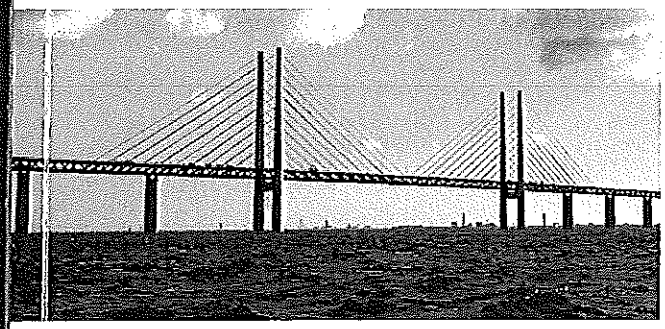
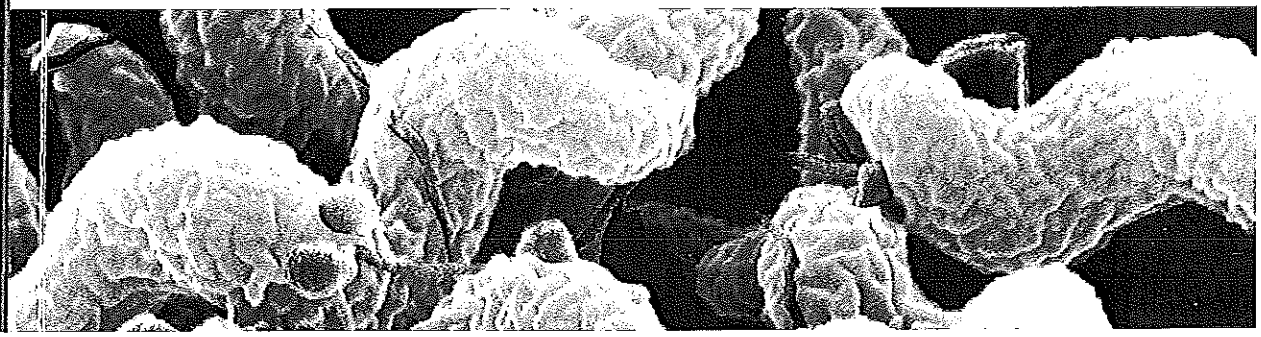


Copenhagen 30th August - 3rd September



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:el plates with grooves

ficult to clean and disinfect. On
uding *Listeria* spp. In this study,
of 0.5, 0.2, 1, 2 or 5 mm. The
exposed to chlorine (Suma Tab
nonium compound, 740 mg/l)
pensions of milk, ham, smoked
and dry (Rh=50%) conditions.
old, mixed biofilms at 15°C, in

ied with Qac, while the use of
300 mg/L gave reductions of
nd 2-4 log units for chlorine.
og factor 2. The reduction of
: reductions were obtained (4
ly). The reduction of the test
s, and in the shallow grooves
vith the same concentrations
re not resistant. Probably the
ıize accumulation of *Listeria*
ıment, incorporated with ef-

and eating food conta-
ıccur through contacting
mperfect and/or irregular
ome unfavourable con-
ates, industry isolates,
to grow and form bio-
/ater with 0.05 % or 1
ystal violet. *LuxS*, gene
m formation process,
nplementary to the *luxS*
xS by polymerase chain
A subunit of topoisome-
:ernalin protein), provi-
ıplicon needed 50 cycles
ained in all strains – in
nt 3 or 4 fragment sets,
nined strains different
t sequence. Two *luxS*
conditions.

PEB2.36 **Hyper- and hypo-biofilm forming mutants of *Listeria monocytogenes* G (Serotype 4b)**

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The foodborne pathogen *Listeria monocytogenes* is a serious public health concern. The serotype 4b strains account for about 40% of the sporadic cases and the majority of the outbreaks. Some *Listeria monocytogenes* form biofilm and this is a cause of concern for the food industries. This study was undertaken in order to improve our understanding of the biofilm formation by identifying genes associated with hyper- or hypoformation. **Methods:** A library of more than 2000 Tn917 insertion mutants was constructed by insertion of transposon Tn917 in *L. monocytogenes* G (serotype 4b) chromosome DNA. Biofilm-formation mutants were identified by use of a microtiter assay employing 1% crystal violet. The insertion sites of Tn917 were found by inverse PCR and BLAST analysis, and the genes responsible for changes in biofilm-formation were identified. Those genes were knocked out by homologous recombination to study the function and mechanism in biofilm formation using proteomics technique. **Results:** After screening a subset of the library 1 positive and 10 negative biofilm-fomation mutants were found. Four genes were identified. One associated with hyperproduction lm.G_1771, and three with decreased production, gltB (lm.G_1758), lm.G_1497, lm.G_2324, encoding an ABC transporter-permease, glutamate synthase, a MerR family transcriptional regulator, and a conserved hypothetical protein, respectively. After further studies, we found that the *dlt* operon encoding the enzymes incorporating D-ala residues into lipoteichoic aceids (LTAs) was strongly down-regulated in the positive mutant. Moreover, the mutant was found to be more sensitive to some membrane active antimicrobials after using a standardized MICs microdilution assay. One of the biofilm negative mutants, the *gltB* insertion mutant, was found to be sensitive to oxidative stress and the inactivition of its upstream gene which encodes a LysR family transcriptional regulator could also cause a decrease in biofilm formation.

PEB2.37 **Response to abiotic stresses in *Oenococcus oeni PSU-1*: a whole-transcriptome view**

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Oenococcus oeni is the most important bacterial species for winemaking, as strains of this *taxon* are the principal actors of malolactic fermentation (MLF). MLF follows alcoholic fermentation and is highly desired in certain styles of wines, both white and red, as it confers positive characteristics in the sensory quality and improves their microbial stability.
Scarceness of nutrients, acidic pH, high ethanol content and other stress factors make wine a hostile environment for bacterial growth. However, *O. oeni* is a lactic acid bacterium well adapted to this ecological niche and shows several genomic peculiarities, e.g. the lack of the mismatch repair (MMR) system. Those traits make it a very important bacterium not only for applied purposes but also for basic research, and it could be considered a model organism for Gram-positive bacteria besides *B. subtilis* and *L. lactis*.
In the present study a whole transcriptome analysis was performed on the *O. oeni* model strain PSU-1 by using a custom 12k Combimatrix chip with 1741 probes specific for almost all the ORFs and pseudogenes annotated for the strain (http://ddlab.sci.univr.it/FunctionalGenomics/datasheets/Ooeni1.0.html).
The experimental design was aimed at evaluating differential gene expression in comparison to laboratory conditions for optimal growth. Shock conditions tested were presence of 10% v/v ethanol, pH 3.5, and both conditions applied simultaneously, besides heat shock at 42°C and shocks were applied for 6 hours to a mid-exponential grown culture.
Results showed that the major modifications of gene expression were obtained when heat shock and the combination of pH and ethanol were applied, while pH variation appeared to be the most tolerated condition. Interestingly, pseudogenes were expressed in almost all conditions, confirming the suggested hypothesis that they still belong to the stress response machinery of the cell and that they could be functional in other *O. oeni* strains. More in general, the results of this study will have an impact on the comprehension of the general mechanism of stress response in *O. oeni* compared to the model organisms of Gram-positive bacteria, and also in giving useful information to improve procedures of biomass preparation and management of MLF in wine.